I CLAIM:

1. A method for preparing plasmid from host cells, wherein the host cells contain the plasmid, the method comprising:

(a) providing a plasmid solution comprised of unligatable open circular plasmid;

(b) reacting the unligatable open circular plasmid with one or more enzymes and appropriate nucleotide cofactors, such that at least some unligatable open circular plasmid is converted to 3'-hydroxyl, 5'-phosphate nicked plasmid;

(c) reacting the 3'-hydroxyl, 5'-phosphate nicked plasmid with a DNA ligase and DNA ligase nucleotide cofactor, such that at least some 3'-hydroxyl, 5'-phosphate nicked plasmid is converted to relaxed covalently closed circular plasmid; and

(d) reacting the relaxed covalently closed circular plasmid with a DNA gyrase and DNA gyrase nucleotide cofactor, such that at least some relaxed covalently closed circular plasmid is converted to negatively supercoiled plasmid.

2. The method according to claim 1, wherein reaction (b) is performed by incubating with a DNA polymerase in the presence of deoxyribonucleoside triphosphates.

3. The method according to claim 2, wherein the DNA polymerase is DNA polymerase I.

4. The method according to claim 2, wherein reactions (b), (c), and (d) are combined in a single in vitro incubation, by incubating with a mixture comprising a DNA polymerase, DNA ligase, and DNA gyrase.

5. The method according to claim 4, wherein the mixture further comprises a kinase enzyme, wherein said kinase enzyme converts the nucleotide by-product of DNA gyrase nucleotide cofactor back to nucleotide cofactor in the presence of a high energy phosphate donor.

6. The method according to claim 4, wherein the plasmid solution further comprises linear chromosomal DNA and the mixture further comprises one or more exonuclease(s), wherein the

exonuclease(s) selectively degrade the linear chromosomal DNA without substantially degrading open circular plasmid, relaxed covalently closed circular plasmid, and supercoiled plasmid.

7. The method according to claim 1, wherein reaction (b) is performed with a 3' deblocking enzyme, DNA polymerase, and deoxyribonucleoside triphosphates.

8. The method according to claim 7, wherein the DNA polymerase is DNA polymerase I.

9. The method according to claim 7, wherein reactions (b), (c), and (d) are combined in a single in vitro incubation, by incubating with a mixture comprising a 3' deblocking enzyme, DNA polymerase, DNA ligase, and DNA gyrase.

10. The method according to claim 9, wherein the plasmid solution further comprises linear chromosomal DNA and the mixture further comprises one or more exonuclease(s), wherein the exonuclease(s) selectively degrade the linear chromosomal DNA without substantially degrading open circular plasmid, relaxed covalently closed circular plasmid, and supercoiled plasmid.

11. The method according to claim 7, wherein the 3' deblocking enzyme is selected from the group consisting of exonuclease III, endonuclease IV, 3'-phosphatase, polynucleotide kinase – 3'-phosphatase, and combinations thereof.

12. The method according to claim 1, wherein the plasmid solution further comprises linear chromosomal DNA and the method further comprises (e) reacting the linear chromosomal DNA with one or more exonuclease(s), wherein said exonuclease(s) selectively degrade the linear chromosomal DNA without substantially degrading relaxed covalently closed circular plasmid and supercoiled plasmid.

13. The method according to claim 12, wherein the exonuclease(s) is selected from the group consisting of exonuclease I, exonuclease III, exonuclease V, exonuclease VII, exonuclease VIII, lambda exonuclease, T5 exonuclease, T7 exonuclease, and combinations thereof.

14. The method according to claim 1, wherein reaction (d) results in less than 20% of total plasmid in catenated form.

15. The method according to claim 1, wherein greater than 75% of open circular plasmid in the plasmid solution is converted to supercoiled plasmid by reactions (b), (c), and (d).

16. The method according to claim 1, wherein reaction (b) is performed with 3'-phosphatase and polynucleotide kinase.

17. The method according to claim 16, wherein reactions (b), (c), and (d) are combined in a single in vitro incubation by reacting with a mixture comprising polynucleotide kinase, 3'-phosphatase, DNA ligase, and DNA gyrase.

18. The method according to claim 1, wherein the plasmid solution is produced by preparing a cleared lysate of the host cells.

19. The method according to claim 1, wherein the plasmid solution is produced by preparing a cleared lysate of the host cells and further purifying plasmid from other host cell components.

20. The method according to claim 19, wherein unligatable open circular plasmid in the plasmid solution consists essentially of (i) unligatable open circular plasmid which was present in the host cells prior to cell lysis, (ii) supercoiled plasmid in the host cells which was unintentionally converted to unligatable open circular plasmid during preparation of the cleared lysate, (iii) supercoiled plasmid in the cleared lysate which was unintentionally converted to unligatable open circular plasmid by further purification of plasmid from other host cell components, or (iv) a combination thereof.

21. The method according to claim 1, wherein the plasmid solution further comprises supercoiled plasmid and reactions (b), (c), and (d) are performed (i) without prior purposeful

conversion of the supercoiled plasmid to linear form, (ii) without prior purposeful conversion of supercoiled plasmid to open circular plasmid, (iii) without prior purposeful conversion of supercoiled plasmid to relaxed covalently closed circular plasmid, and (iv) without prior purposeful conversion of open circular plasmid of (a) to single stranded circular DNA.

22. The method according to claim 1, wherein reactions (b), (c), and (d) are performed without in vitro plasmid replication and without prior in vitro plasmid replication.

23. The method according to claim 1 further comprising preparing a cleared lysate of the host cells, wherein the cleared lysate comprises the unligatable open circular plasmid.

24. The method according to claim 1, wherein the unligatable open circular plasmid was synthesized by the host cells.

25. The method according to claim 1, wherein the plasmid solution does not comprise purposefully in vitro synthesized, unligatable open circular plasmid.

26. The method according to claim 1, wherein the plasmid solution further comprises supercoiled plasmid and reactions (b), (c), and (d) are performed without purposeful in vitro conversion and without prior purposeful in vitro conversion of (i) the supercoiled plasmid to an undesired form, and (ii) open circular plasmid to an undesired form.

27. The method according to claim 1, wherein the plasmid solution further comprises supercoiled plasmid and reactions (b), (c), and (d) are performed without prior purposeful separation of open circular plasmid from supercoiled plasmid.

28. The method according to claim 1 further comprising recovering the supercoiled plasmid after reaction (d).

29. The method according to claim 28 further comprising transforming the recovered plasmid into recipient cells.

30. A method for preparing plasmid from host cells, wherein the host cells contain the plasmid, the method comprising:

(a) providing a plasmid solution comprised of unligatable open circular plasmid;

(b) reacting the unligatable open circular plasmid with one or more enzymes and appropriate nucleotide cofactors, such that at least some unligatable open circular plasmid is converted to 3'-hydroxyl, 5'-phosphate nicked plasmid;

(c) reacting the 3'-hydroxyl, 5'-phosphate nicked plasmid with a DNA ligase in the presence of DNA ligase nucleotide cofactor, such that at least some 3'-hydroxyl, 5'-phosphate nicked plasmid is converted to relaxed covalently closed circular plasmid; and

(d) reacting the relaxed covalently closed circular plasmid with a reverse DNA gyrase and reverse DNA gyrase nucleotide cofactor, such that at least some relaxed covalently closed circular plasmid is converted to positively supercoiled plasmid.

31. A method for preparing plasmid from host cells, wherein the host cells contain the plasmid, the method comprising:

(a) providing a plasmid solution comprised of unligatable open circular plasmid and supercoiled plasmid;

(b) reacting the unligatable open circular plasmid with one or more enzymes and appropriate nucleotide cofactors, such that at least some unligatable open circular plasmid is converted to 3'-hydroxyl, 5'-phosphate nicked plasmid;

(c) reacting the 3'-hydroxyl, 5'-phosphate nicked plasmid with a DNA ligase and DNA ligase nucleotide cofactor, such that at least some 3'-hydroxyl, 5'-phosphate nicked plasmid is converted to relaxed covalently closed circular plasmid, wherein the relaxed covalently closed circular plasmid is not further converted enzymatically in vitro to supercoiled plasmid; and

(d) recovering the supercoiled plasmid and the relaxed covalently closed circular plasmid;

wherein reactions (b) and (c) are performed without purposeful in vitro conversion and without prior purposeful in vitro conversion of (i) the supercoiled plasmid to an undesired form, and (ii) open circular plasmid to an undesired form; and

wherein reactions (b) and (c) are performed without prior purposeful separation of unligatable open circular plasmid from supercoiled plasmid.

12. The method according to claim 31, wherein the plasmid solution further comprises linear chromosomal DNA and the method further comprises (e) reacting the linear chromosomal DNA with one or more exonuclease(s), wherein said exonuclease(s) selectively degrade the linear chromosomal DNA without substantially degrading relaxed covalently closed circular plasmid and supercoiled plasmid.

33. The method according to claim 31 further comprising transforming the recovered plasmid into recipient cells.

34. An enzyme composition useful for converting unligatable open circular plasmid to supercoiled plasmid comprising: 3' deblocking enzyme, DNA polymerase, DNA ligase, and DNA gyrase.

35. The composition according to claim 34 further comprising a kinase enzyme, wherein said kinase enzyme converts the nucleotide by-product of DNA gyrase nucleotide cofactor back to nucleotide cofactor in the presence of a high energy phosphate donor.

36. The composition according to claim 34 further comprising one or more exonuclease(s), wherein the exonuclease(s) selectively degrades linear chromosomal DNA without substantially degrading relaxed covalently closed circular plasmid and supercoiled plasmid.

37. The composition according to claim 36, wherein the exonuclease(s) does not substantially degrade open circular plasmid.

38. The composition according to claim 34, wherein the 3' deblocking enzyme is selected from the group consisting of exonuclease III, endonuclease IV, 3'-phosphatase, polynucleotide kinase – 3'-phosphatase, and combinations thereof.

39. An enzyme composition useful for converting unligatable open circular plasmid to supercoiled plasmid comprising: DNA polymerase, DNA ligase, DNA gyrase, and one or more exonuclease(s); wherein the exonuclease(s) selectively degrades linear chromosomal DNA without substantially degrading relaxed covalently closed circular plasmid and supercoiled plasmid.

40. The composition according to claim 39, wherein the exonuclease(s) does not substantially degrade open circular plasmid.

41. An enzyme composition useful for converting unligatable open circular plasmid to supercoiled plasmid comprising: DNA gyrase, DNA ligase, polynucleotide kinase, and 3'-phosphatase.

42. A kit for converting unligatable open circular plasmid to supercoiled plasmid comprising: (a) DNA polymerase, (b) DNA ligase, (c) DNA gyrase or reverse DNA gyrase, and (d) 3'-deblocking enzyme and/or exonuclease in one or more containers.

43. A kit for converting unligatable open circular plasmid to supercoiled plasmid comprising: (a) DNA polymerase, (b) DNA ligase, (c) DNA gyrase, and (d) instructions to practice the method according to claim 1.

44. A kit for converting unligatable open circular plasmid to supercoiled plasmid comprising: (a) DNA polymerase, (b) DNA ligase, and (c) instructions to practice the method according to claim 31.